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PRECLINICAL RESEARCH

Prior Exercise Training Improves the Outcome of Acute Myocardial Infarction in the Rat

Heart Structure, Function, and Gene Expression

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OBJECTIVES The aim of this research was to investigate the structural, functional, and molecular features of the remodeling heart in prior swim-trained infarcted rats.

BACKGROUND Physical exercise training is a known protective factor against cardiovascular morbidity and mortality. The structural and molecular aspects underlying this protection in the remodeling heart have not been investigated.

METHODS After seven weeks of swimming exercise training, rats underwent surgical ligation of the left coronary artery followed by a four-week sedentary period. Untrained control rats underwent the same surgical protocol. Left ventricular function was assessed by echocardiography four weeks after infarction, and hearts were sampled for histological and molecular analysis. Ribonucleic acid from the surviving left ventricle was analyzed by complementary deoxyribonucleic acid arrays followed by Northern blotting or quantitative reverse transcription polymerase chain reaction of selected messenger ribonucleic acids (mRNAs).

RESULTS Scar area was 1.6-fold smaller ($p = 0.0002$), arteriolar density was 1.7-fold higher ($p = 0.0002$), and left ventricular shortening fraction was 1.9-fold higher ($p = 0.003$) in the exercise-trained compared with sedentary hearts. Eleven genes whose expression level varied by at least ±1.5-fold distinguished the prior exercised rats from their sedentary counterparts. Compared with sedentary, the exercised hearts displayed 9- and 2.4-times lower levels of atrial natriuretic peptide and aldolase mRNA ($p = 0.03$ and 0.04, respectively), and a 2.7- and 1.9-fold higher abundance of cytochrome c-oxidase and fatty acid binding protein, respectively ($p < 0.03$, each).

CONCLUSIONS Swimming exercise training before acute myocardial infarction reduces scar size, increases arteriole density, and manifests adaptation of stress- and energy-metabolism-related genes that may contribute to the improved heart function observed during remodeling. (J Am Coll Cardiol 2005;45:931–8) © 2005 by the American College of Cardiology Foundation

A sedentary lifestyle has been associated with increased risk for cardiovascular morbidity and mortality (1–3). Regular physical activity and improved functional capacity are associated with reduced incidence of coronary events and frequency of fatal myocardial infarctions (MI) (2–5).

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Aerobic exercise training increases left ventricular (LV) mass, augments the cardiac index and stroke volume, and decreases the resting heart rate (6). Cellular adaptations of the exercised myocardium include higher mitochondrial number, increased myocyte contractility and calcium sensitivity, along with increased activity and expression of related enzymes that contribute to the improved heart function (7–10).

Several studies reported on improved response of exercised hearts to injurious insults such as ischemia-reperfusion or acute MI shortly after the insult. When preceding ischemia-reperfusion, both a single bout of exercise and long-term training reduced the resultant infarct and ameliorated the impaired function of rat and dog hearts (11–14). Enhanced expression and activation of antioxidant and heat shock proteins were implicated, as well as involvement of mitochondrial potassium channels (11–14). In a rat model of acute MI, swimming exercise training performed before irreversible coronary artery occlusion reduced the infarct area as examined at the subacute phase two days after infarction (15). No additional studies are known to us reporting diminution of infarct size due to prior exercise training in either the acute or the chronic healing phase.

In light of the positive effects of physical activity on heart function, the extent of damage developed early after infarction, and the frequency of fatal heart disease, we hypothe-

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Abbreviations and Acronyms

ANP	= atrial natriuretic peptide
COX	= cytochrome c-oxidase
Ex	= exercise
LV	= left ventricle/ventricular
LVEDD	= left ventricular end-diastolic diameter
LVESD	= left ventricular end-systolic diameter
MI	= myocardial infarction
mRNA	= messenger ribonucleic acid
qRT-PCR	= quantitative reverse transcription polymerase chain reaction
Sed	= sedentary
SF	= shortening fraction

sized that the favorable effects of prior exercise on cardiac injury sustain beyond the acute phase. We tested whether any impact of exercise training conducted before acute MI upholds to the remodeling phase. We found reduced infarct size, increased arteriole density, better LV function, and favorable alterations in gene expression four weeks after infarction in prior swim-trained rats.

METHODS

The experimental design. The study conformed to the "Guide for the Care and Use of Laboratory Animals" published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was approved by the Institutional Committee for Animal Care and Use at Tel-Aviv University. Male Sprague-Dawley rats (250 to 290 g) were assigned to exercise or sedentary groups. The exercise rats underwent a daily swimming session six days a week for seven weeks. The first session was for 15 min, and duration increased by 15 min each session reaching 90 min on the sixth day and maintained so to the end of training. At seven weeks, exercising and sedentary animals were randomized into non-infarcted (Sed, Ex) or infarcted groups (SedMI, ExMI). After MI induction the rats were kept for four weeks healing with no additional exercise (a total of 11 weeks) as were the non-infarcted controls, Ex and Sed.

Infarction, echocardiography, and sacrifice. The left anterior descending coronary artery of anesthetized (intramuscular, xylazine, 10 $mg \cdot kg^{-1}$, and ketamine, 90 $mg \cdot kg^{-1}$) and ventilated (Harvard respirator, 2.5 ml, 75 to 78 $strikes \cdot min^{-1}$) rats was permanently ligated with a 5/0 silk thread. Transthoracic echocardiography was performed three to four days before sacrifice on a subgroup of the infarcted animals using a 12-MHz transducer (Sonos-5500, Hewlett-Packard, Andover, Massachusetts). M-mode tracing of the LV was obtained from the parasternal long-axis view to measure left ventricular end-diastolic diameter (LVEDD) and left ventricular end-systolic diameter (LVESD), and shortening fraction (SF) (%) was calculated.

All animals were sacrificed 11 weeks after the initiation of training, and hearts were harvested using either perfusion fixation with 10% buffered formalin (16) under constant pressure (80 mm Hg), or quick removal, weighing, dissecting the viable LV free of scar and right ventricle, then freezing it in liquid nitrogen for storage at −70°C until analyzed.

Histology and morphometry. INFARCT AND MUSCLE DIMENSIONS. Two 5-μm sections taken at 5 and 6 mm from the apex were stained with Masson trichrome. Scar area, LV muscle area, and thickness of the interventricular septum were measured in each section applying a Zeiss Axioscope-II microscope (Zeiss, Jena, Germany) and computerized planimetry. Values obtained from the two sections were averaged.

BLOOD VESSEL COUNT. Sections were stained with anti-smooth muscle alpha-actin (mouse monoclonal, Sigma, St. Louis, Missouri), diluted 1:4000, and visualized with the Dako EnVision kit (Dako, Glostrup, Denmark). Positively stained blood vessels were counted in nine non-overlapping randomly selected fields within the non-infarcted LV at ×400 magnification using an Olympus BX52 microscope (Olympus, Tokyo, Japan) (17).

RNA analysis. Total ribonucleic acid (RNA) was extracted from frozen samples of the non-infarcted LV (free of the scar and right ventricle) employing a previously described procedure (18). From every experimental group, four randomly selected RNA samples were pooled, each sample (5 μg) representing a different heart. Each pool (20 μg of total RNA) was analyzed on complementary deoxyribonucleic acid (cDNA) expression arrays containing 1,176 rat-specific probes (Rat 1.2, Clontech, Palo Alto, California). The RNA preparation for the array analysis was as specified by the manufacturer. In brief, traces of genomic DNA were removed by incubating the pooled RNA with RNase-free DNase I (Clontech), followed by phenol-chloroform extraction and ethanol precipitation. Aliquots (1 μg) of the recovered RNAs were reverse-transcribed in complementary DNA Synthesis Mix (Clontech) containing α-[^{32}P]-dATP. Each ^{32}P-labeled cDNA was hybridized to a membrane cDNA-array according to the manufacturer's instructions. Hybridization signals, acquired by a Cyclone phosphorimager (Packard-PerkinElmer, Downers Grove, Illinois), were calculated with the Clontech AtlasImage 2.01-software, and the result for each transcript was expressed as percent of the global hybridization signal. For every RNA pool, three repetitive hybridizations were performed to minimize false signals, and the three results obtained for each gene were averaged to give an expression score. Northern blot analysis was performed loading 10 μg RNA per lane and normalizing to 18S ribosomal RNA, using previously described probes and procedures (18). For reverse transcription, 0.5 μg RNA was incubated with 50 U RT-Superscript II (Gibco-BRL, Gaithersburg, Maryland) in the Superscript-buffer and 2 μM oligo-$dT_{12\text{-}18}$ (Sigma), 0.1 mM dithiothreitol, 200 μM dNTPs, and 40 U RNAguard (Gibco-BRL). Quantitative polymerase chain reaction was performed in the RotorGene DNA Amplification System (Corbett Research, Australia, courtesy of Tamar Ltd., Israel) using primers specified by Clontech (0.5

Table 1. Body and Heart Weight

	SedMI (n = 20)	Sed (n = 10)	ExMI (n = 23)	Ex (n = 9)
BW (g)	379 ± 21	381 ± 28	388 ± 17	402 ± 29
HW (mg)	1,435 ± 220*	1,157 ± 63	1,398 ± 170†	1,234 ± 122
HW/BW (mg/g)	3.79 ± 0.61*	3.04 ± 0.14	3.59 ± 0.41†	3.07 ± 0.25

Mean ± SD. *p < 0.001 vs. Sed; †p < 0.01 vs. Ex.
BW = body weight; Ex = exercise-trained; ExMI = exercised-infarcted; HW = heart weight; HW/BW = the ratio of heart weight-to-body weight; MI = myocardial infarction; Sed = sedentary; SedMI = sedentary-infarcted.

μM each) in a 20 μl reaction mixture containing 200 μM dNTPs, 2.5 mM $MgCl_2$, 5 U Taq Polymerase (Bioline, London, United Kingdom) and SYBR-Green (Amresco, Solon, Ohio). Results for each mRNA were normalized to the beta-actin mRNA.

Calculations and statistics. Heart functional and histological data as well as RNA scores obtained from Northern analysis and quantitative reverse transcription polymerase chain reaction (qRT-PCR) were compared by single factor analysis of variance followed by Student *t* test. A p value <0.05 was considered statistically significant.

The cDNA arrays were calculated and analyzed statistically as follows: studying the distribution of the signal intensity readings revealed three salient features of the data.

1. Highly skewed marginal distributions.
2. Nonlinear dependence of the mean of the between-hybridization intensity differences on hybridization intensity sums.
3. Dependence of the variance of the between-hybridization intensity differences on hybridization intensity sums.

Problems 1 and 3 were solved by a −0.50 power transformation (i.e., $r_i \rightarrow -1/\sqrt{r_i}$). To overcome problem 2, the transformed readings were normalized by an intensity-dependent normalization (19). The normalized spot readings were then averaged within each treatment group. The Z-scores for comparing treatments were attained by computing the between-treatment differences and dividing them by a robust estimator of the between-treatment standard deviation.

RESULTS

Mortality. No difference was observed in postoperative mortality between exercised and sedentary rats (14.3% and 20.7% in Ex and Sed, respectively, p = NS, chi-square test). No mortality was registered beyond the first 24 h.

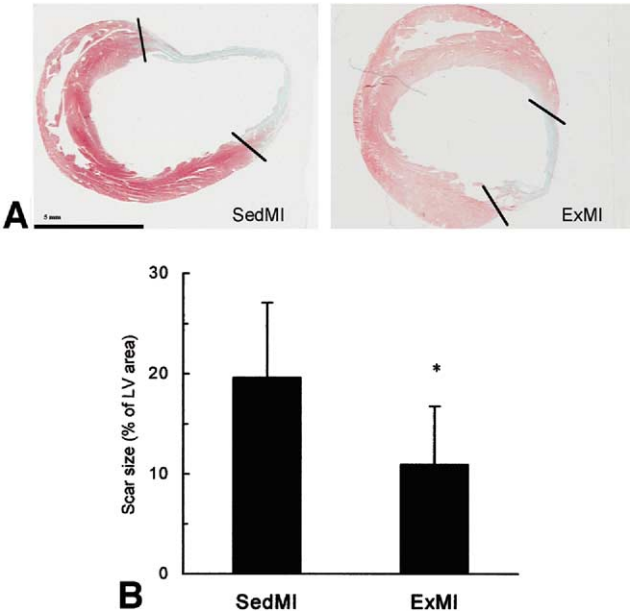

Figure 1. Measurements of scar size. **(A)** Representative transversal sections stained with Masson trichrome; **bars** = scar borders. **(B)** Relative scar area expressed as percent of left ventricular (LV) cross-sectional area. Values expressed as mean ± SD. *p = 0.00007 vs. SedMI; SedMI, n = 15; ExMI, n = 14. ExMI = exercised-infarcted; MI = myocardial infarction; SedMI = sedentary-infarcted.

Heart and body weight. The initial body weight of sedentary and exercising animals was similar (279 ± 25 and 268 ± 18 in Sed and Ex, respectively). Four weeks after MI, the body weight of all animals was alike (Table 1), whereas the heart weight and the ratio heart-weight-to-body-weight of SedMI and ExMI rats were both significantly higher than corresponding values in their non-infarcted counterparts. No difference in either heart weight or the ratio heart-weight-to-body-weight was observed between the Ex and Sed rats (Table 1).

LV morphology and function. As shown in Table 2, the cross-sectional area of the scar was significantly smaller in ExMI compared with SedMI (by 40%), whereas area of the viable muscle, and thickness of the interventricular septum were both significantly larger in ExMI, by 23% and 9%, respectively. Accordingly, the proportion of the scar within the LV cross-sectional area was smaller by 44% in ExMI (Fig. 1), implicating differences in postinfarct remodeling in

Table 2. Histological Measurements

	Scar Area (mm^2)	Muscle Area (mm^2)	IVS Thickness (mm)	Blood Vessels (Count/mm^2)
SedMI	6.7 ± 2.4 (15)	28.2 ± 10.1 (15)	1.54 ± 0.44 (15)	5.71 ± 1.08 (6)
ExMI	4.1 ± 1.9* (14)	34.8 ± 10.9* (14)	1.68 ± 0.46† (14)	9.71 ± 1.09*§ (5)
Sed	—	—	1.55 ± 0.32 (5)	6.28 ± 1.2 (5)
Ex	—	—	2.07 ± 0.32‡ (3)	6.13 ± 2.97 (4)

Measurements in transversal heart sections stained with Masson trichrome for fibrous tissue and with an antibody against vascular smooth muscle alpha-actin to label arteriolar blood vessels. Mean ± SD. *p = 0.0002 vs. SedMI; †p = 0.05 vs. SedMI; ‡p = 0.01 vs. Sed; §p = 0.04 vs Ex. Numbers of hearts are in parentheses.
IVS = interventricular septum; other abbreviations as in Table 1.

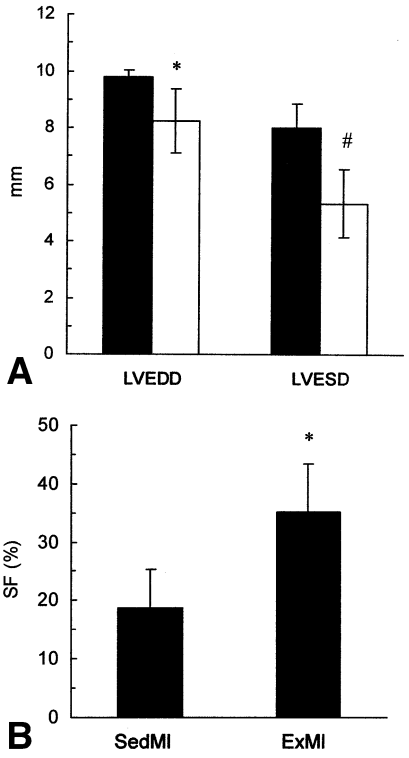

Figure 2. Measurements of left ventricular function. **(A)** Left ventricular end-systolic diameter (LVESD) and left ventricular end-diastolic diameter (LVEDD) were measured by echocardiography four weeks after myocardial infarction. **(B)** Left ventricular shortening fraction (SF [percent]) in the same hearts. Values expressed as mean ± SD. *p = 0.003; #p = 0.001 vs. SedMI. In **panel A**, **solid bar** = SedMI and **open bar** = ExMI. ExMI = exercised-infarcted (n = 8); SedMI = sedentary-infarcted (n = 4).

these hearts. Thickness of the interventricular septum was similar in either SedMI and Sed or ExMI and Ex but was significantly larger in each of the exercising groups compared with its sedentary counterpart (Table 2), indicating hypertrophy in the non-infarcted myocardium of the prior trained rats.

The density of arterioles, the blood vessels positive for smooth muscle alpha-actin, was significantly higher in ExMI hearts compared with either SedMI (by 70%, Table 2) or the two non-infarcted controls (by ~50%, Table 2). It is, therefore, suggested that prior exercise training potentiated post-MI arteriogenesis specifically in ExMI hearts.

Complying with the morphological differences, higher LVESD and LVEDD were measured in SedMI hearts (Fig. 2A), indicating poorer LV contraction and greater LV dilatation. Left ventricular SF was 1.9-fold higher in the ExMI group (Fig. 2B), signifying improved LV function compared with SedMI.

Gene expression profile. To examine whether the modified structure and function of ExMI hearts correspond to variations in gene expression, we conducted a multitranscript screening using complementary DNA arrays followed by quantification of transcripts of interest employing alternative methods for RNA determination. As

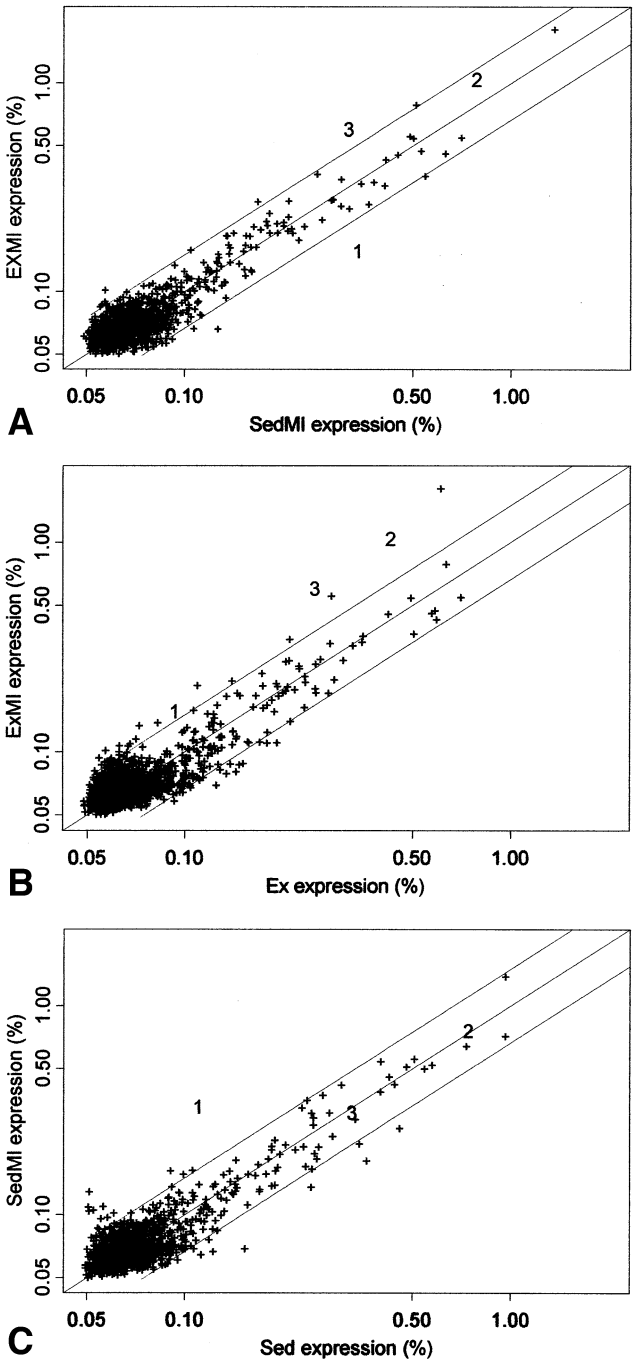

Figure 3. Relative gene expression deduced from cDNA arrays. Scatter plots comparing **(A)** exercised-infarcted (ExMI) with sedentary-infarcted (SedMI). **(B)** Exercised-infarcted with Ex. **(C)** Sedentary-infarcted with Sed. The score for each gene is expressed as percent of the global expression. The **middle diagonal** marks the line of identity and the **upper and lower diagonals** outline the range of 1.5-fold change. The numbers denote the positions of atrial natriuretic peptide **(1)**, cytochrome c-oxidase (COX) subunit 6A **(2)**, and COX subunit 8h **(3)**.

shown in Figure 3, expression signal of most analyzed genes was below 0.1% of the global expression measured. To ensure exclusion of false positive signals, we focused on the 151 genes whose expression level was above the

Table 3. Differentially Expressed Genes

	Gene	GenBank	Relative Expression
(a) ExMI vs. SedMI	Cytochrome-c-oxidase 6A (2)	X12554	↑
	Cytochrome-c-oxidase 8h (3)	X64827	↑
	Cytochrome-c-oxidase 5B	D10952	↑
	ATPase, calcium (SERCA-2)	J04022	↑
	HMG-coA synthase	M33648	↑
	GM-CSF	U00620	↑
	40S ribosomal protein S11	K03250	↑
	ANP (1)	X01118	↓
	Glutamate receptor GLUR-4	M85037	↓
	TGF-β masking protein	M55431	↓
	Cytochrome-c-oxidase, Sertoli	S79304	↓
(b) ExMI vs. Ex	ANP (1)	X01118	↑
	Cytochrome-c-oxidase 6A (2)	X12554	↑
	Cytochrome-c-oxidase 8h (3)	X64827	↑
	VEGP2	X74806	↑
	40S ribosomal protein S17	K02933	↑
	GCSF	U37101	↑
	GM-CSF	U00620	↑
	Tyrosine phosphatase, nuclear	L27843	↓
	ATP synthase 5B, mitochondrial	M19044	↓
	ATP synthase 5F1, mitochondrial	M35052	↓
	ATPase, calcium (SERCA-2)	J04022	↓
	Na/K ATPase, beta subunit	J02701	↓
	Creatine kinase, mitochondrial	X59737	↓
	CNP	D90219	↓
	PFK muscle	U25651	↓
	Triacylglycerol lipase (hepatic)	M16235	↓
(c) SedMI vs. Sed	ANP (1)	X01118	↑
	IL-13	L26913	↑
	Prostaglandin receptor F2a	X16956	↑
	Cyclin-G1	X70871	↑
	PDGF associated protein	U41744	↑
	Protymosin alpha	M20035	↑
	GM-CSF	U00620	↑
	ATPase, calcium (SERCA-2)	J04022	↓
	H-FABP	J02773	↓
	Spleen-tyrosin kinase	U21684	↓
	VEGF receptor1	D28498	↓
	Sodium channel SCNB2	U37026	↓
	L-type calcium channel	M59786	↓
	CNP	D90219	↓

List of mRNAs displaying ±1.5-fold or more expression difference in the comparisons shown in Figure 3. Numbers in parentheses correspond to the same digits in the scatter plots of Figure 3. ↑ denotes higher expression and ↓ denotes lower expression in the specified comparison.

ANP = atrial natriuretic peptide; CNP = C-type natriuretic peptide; GCSF = granulocyte colony stimulating factor; GM-CSF = granulocyte-macrophage colony stimulating factor; H-FABP = heart fatty acid binding protein; HMG-coA synthase = 3-hydroxy-3-methylglutaryl-CoA synthase; IL-13 = interleukin-13; PDGF = platelet-derived growth factor; PFK = phosphofructokinase; SERCA = sarcoplasmic reticulum Ca-ATPase; TGF = transforming growth factor; VEGF = vascular endothelial growth factor; VEGP = von Ebner's gland protein; other abbreviations as in Table 1.

0.1% threshold (20). Comparisons performed between ExMI and SedMI, ExMI and Ex, and SedMI and Sed (Fig. 3) highlighted 32 genes, whose expression level deviated by at least 1.5-fold in one comparison or more. Eleven differentially expressed genes distinguished ExMI from SedMI hearts (Table 3a), whereas 16 and 14 genes distinguished ExMI and SedMI from their respective non-infarcted controls (Tables 3b and 3c).

Most prominent were the differences observed for the atrial natriuretic peptide (ANP) and subunits 6A and 8h of cytochrome c-oxidase (COX). ANP expression was enhanced in both MI groups, yet its expression in ExMI during remodeling was lower than in SedMI (Figs. 3A to 3C, Tables 3a to 3c). By contrast, MI did not change the transcript level of COX subunits in SedMI hearts (Fig. 3C, Table 3c), whereas, in ExMI hearts, COX mRNAs were elevated compared with both SedMI and Ex (Figs. 3A and 3B, Tables 3a and 3b). The array analysis also indicated reduced expression of sarcoplasmic reticulum calcium ATPase in the two MI groups, but when compared with SedMI, ExMI hearts maintained higher levels of sarcoplasmic reticulum calcium ATPase mRNA (Tables 3a to 3c).

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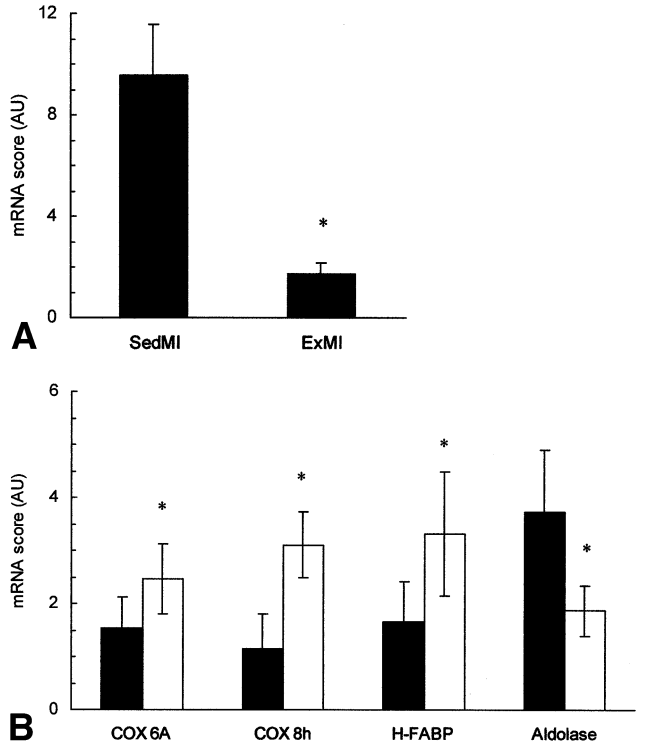

Figure 4. Expression of selected genes. RNA of individual hearts was analyzed for **(A)** atrial natriuretic peptide (by Northern blot hybridization); **(B)** energy metabolism-related genes (by quantitative reverse transcription polymerase chain reaction). Values expressed as mean ± SD; *$p \leq 0.03$ vs. sedentary-infarcted (SedMI); n = 5 in each SedMI and exercised-infarcted (ExMI) group. **Solid bar** = SedMI; **open bar** = ExMI. AU = arbitrary units; COX 6A and COX 8h = subunits of cytochrome C-oxidase; H-FABP = heart fatty acid binding protein.

Expression of selected genes. Expression of ANP and COX subunits 6A and 8h was assessed in the individual specimens to validate the array observations and substantiate the comparison between groups. Consistent with the array results, the abundance of ANP mRNA was nine-fold lower ($p = 0.03$) in ExMI compared with SedMI (Fig. 4A). Also compatible with the array, the levels of COX 6A and 8h mRNAs were both elevated in ExMI compared with SedMI hearts, 1.6-fold ($p = 0.02$) and 2.7-fold ($p = 0.005$), respectively (Fig. 4B). This finding, which suggested higher oxidative capacity in the ExMI hearts, urged us to examine additional energy metabolism-related genes whose array expression exceeded the 0.1% threshold but the differences observed between groups did not surpass the ±1.5-fold change. Employing qRT-PCR, we assessed the heart-specific fatty acid binding protein, involved in fatty acid metabolism towards beta-oxidation and aldolase, active in the anaerobic glycolytic pathway. Compared with SedMI, in ExMI, the abundance of heart-specific fatty acid binding protein mRNA was 1.9-fold higher ($p = 0.01$), and that of aldolase mRNA was 2.4-fold lower ($p = 0.04$) (Fig. 4B), indicating that prior exercise training facilitated aerobic substrate utilization and attenuated the transition to anaerobic energy metabolism during remodeling.

DISCUSSION

Exercise training and cardiac protection. The beneficial effects of exercise training on cardiovascular morbidity have been widely reported, emphasizing the higher survival rates among people conducting physically active life-style but neglecting information of heart performance or infarct size after a coronary event (2–5). Improved heart function and reduced infarct size were both demonstrated in prior exercised animals early after ischemia-reperfusion or acute MI (11–15), overlooking belated time points such as the remodeling phase. We are the first to report the long lasting beneficial effects of prior exercise training in chronically infarcted myocardium that persist even after physical activity was discontinued upon MI surgery.

Heart structure. Exercise training leads to physiological hypertrophy (7), whereas infarction injury elicits compensatory hypertrophy in the noninfarcted myocardium (21). Our finding of a thicker interventricular septum in the two exercised groups, infarcted and non-infarcted, is a sign of hypertrophy that is independent of the infarction injury and suggests prior training and not MI as the cause for septum enlargement. The differences observed in septum thickness, like those found in scar and muscle dimensions, were, however, not reflected in the global heart weights. Within each comparison, ExMI with SedMI and Ex with Sed, similar heart weights were measured, indicating that weighing the fresh tissue was not as informative as analyzing the histological preparations, particularly when hearts were harvested after four weeks detraining, which could diminish hypertrophy (22).

Over two decades ago, McElroy et al. (15) demonstrated diminution of infarct area two days after coronary occlusion in five-week swim-trained rats. Our study extends these findings by showing a 40% reduction in scar size in the same model of prior swim-trained rat, yet four weeks after MI, at the chronic healing phase. McElroy et al. (15) proposed increased myocardial vascularity as a basis for primary injury restriction because exercised non-injured hearts showed a 30% increase in the blood-vessel-to-muscle-fiber ratio. We found a significantly higher arteriolar density exclusively in the ExMI hearts and no difference between the Ex and Sed groups, suggesting that prior exercise training conditioned the myocardium for enhanced arteriogenesis in response to MI. The additional arterioles may have originated from vessels grown during the training period as suggested by McElroy et al. (15), or after the MI as found recently in infarcted myocardium of rats preconditioned by hypoxia-reoxygenation in vivo (23), or else, a combination of the two. A 25% increase in arteriole density and a two-fold rise in the regional ventricular blood flow were reported three weeks after MI in rats preconditioned by hypoxia and reoxygenation (23). Although blood flow was not measured in our experiments, it is likely that a 70% increase in arteriolar density permitted a higher blood flow in the

ExMI hearts that could help preserve the surviving myocardium in these hearts.

Heart function. Improved cardiac function has so far been demonstrated in pre-trained rats subjected to injury due to transient ischemia and subsequent reperfusion (13,14). Our results show that prior exercise training ameliorates the function of chronically infarcted hearts. The finding of a smaller LVEDD indicates reduced chamber dilation, as would be expected when a scar is small, whereas smaller LVESD designates improved myocardial contraction, consistent with a larger muscle area and higher arteriolar density. In addition to the obvious beneficial effects of the structural changes, the superior performance of ExMI hearts might have gained from functional capabilities acquired during the adaptation to exercise training (7–10) and persisted in the viable myocardium throughout post-MI remodeling.

The mechanisms by which exercise training preconditions the heart are not fully understood. In case a transient hypoxia takes place during exercise, ischemic preconditioning may be involved although supportive evidence is lacking. By contrast, early and late cardioprotection were both obtained in exercising dogs in the absence of intermittent ischemia (12). Furthermore, the adaptive changes induced by swimming exercise training differ from those evoked by hypoxia as they involve increased mitochondria-to-fibril ratio and improved contractility that have not been found in the hypoxic rats (7).

Differences in expressed genes. After MI, the surviving myocardium undergoes extensive remodeling and gene reprogramming in every component of the tissue (24,25). To identify genes whose expression during remodeling was altered due to prior exercise training, we employed a gene-array approach. In each, the exercised or non-exercised hearts, different subsets of MI-regulated genes were highlighted, a possible reflection of variation in either the primary response to MI, the progression of post-MI remodeling, or, in the case of the exercised rats, the effect of exercise discontinuation (22).

Of special interest were genes that distinguished SedMI from ExMI hearts. The natriuretic peptide ANP, a fetal cardiac protein, is re-induced in the ventricles under pressure overload, hypertrophy, and heart failure, in correlation with the hemodynamic load and the severity of heart malfunction (26–28). In contrast with pathological overload, the volume load generated by exercise training does not enhance ANP expression (10), and may even diminish the low amount of ANP mRNA that is normally present in the LV (29). In agreement with previous studies (27,28), we found enhanced ANP expression in all of the MI hearts, but the ExMI group displayed significantly lower levels of ANP transcripts, indicative of a lower wall stress as would be expected because the scar is smaller and the muscle fraction is larger in these hearts (27). Thus, the lower ANP expression in ExMI hearts is likely an outcome of improved myocardial preservation in the prior exercised hearts.

After MI, the myocardium shifts from aerobic to anaerobic substrate utilization, and the amount of mitochondrial-encoded transcripts declines, including those encoding COX subunits (30,31). Unlike MI, exercise training up-regulated both cytochrome-oxidase and sarcoplasmic reticulum calcium ATPase (9). The array analysis showed that three COX subunits, a nuclear encoded and two mitochondrial encoded, were expressed at higher levels in ExMI hearts, of which two subunits were further assessed to confirm the array result. Supplementary to the COX gene expression was our finding of higher heart fatty acid binding protein and lower aldolase expression in ExMI hearts that supports a higher capacity for oxidative substrate utilization and a smaller shift to anaerobic energy metabolism. Preservation of aerobic energy metabolism due to prior exercise training apparently contributed to the improved LV function and the restricted myocyte remodeling in ExMI hearts. However, a possibility that the higher capacity for aerobic substrate utilization is secondary to myocyte preservation because of attenuated scar expansion or reduced interstitial remodeling cannot be ruled out (30,31).

Another indication of conserved contractile capacity in ExMI hearts might be the higher levels of sarcoplasmic reticulum calcium ATPase mRNA. Reduction in sarcoplasmic reticulum calcium ATPase after MI was correlated with impaired muscle contractility (32), and its up-regulation with physical exercise after MI was associated with improved contractility (33).

Study limitations. The commercial array we were using contained probes of broad interest not specifically selected for the cardiac phenotype. This relatively low cost array offered a good selection of genes expected to be involved in the structural and functional changes we observed. Contrary to our expectations, the array analysis did not provide evidence for regulation of genes that mark hypertrophy, angiogenesis, fibrosis, oxidative stress, or apoptosis. In part, this may be due to the limited repertoire of probes, the rigorous conditions we defined for the analysis, the use of a single RNA pool for each experimental group, or to sensitivity limitation as illustrated in the case of heart fatty acid binding protein and aldolase. Differences in their expression levels on the arrays failed to exceed the ± 1.5-fold threshold, but were clearly demonstrated with qRT-PCR. It is important to note, however, that there was no contradiction between the qRT-PCR and the array results, as both tools indicated the same direction of changes in the gene expression patterns. Thus, the array analysis served as a screening tool to identify genes of interest while conclusions were drawn from comparisons based on mRNA quantification in each of the individual hearts.

Sampling the hearts four weeks after infarction did not allow, presumably, the detection of earlier events in the response to MI that could be fundamental in restricting the damage. Transcripts potentially involved in these early events, such as regulators of cell death, survival, and even regeneration, might not be detected four weeks after the

injury. Future research should identify these early events, trace their progression, and explain the persistence of the training conditioning four weeks after MI in the absence of further exercise.

Clinical implications. The demonstration that swimming exercise training, performed before acute MI, effectively reduces infarct size and LV dilatation, and improves LV function as late as four weeks after the infarction, along with the corresponding alterations in load-related and oxidative metabolism-related genes, provides new insights of how physical activity reduces the damage of an irreversible coronary occlusion, reinforcing the importance of a physically active life-style in the protection against acute MI.

Conclusions. Prior exercise training in the rat improves the tolerance of acute MI as it restricts LV remodeling and ameliorates the healing process. The smaller scar, larger muscle area, and higher arteriole density provide structural evidence for altered remodeling that suggests lower hemodynamic load, higher contractile capacity, and better blood perfusion. These, and corresponding modifications in gene expression collectively improve LV function in the infarcted prior-exercised hearts.

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